EAST Search History

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	14933	protein-protein adj interaction	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2007/05/11 16:15
L7	32	l1 with ((protein adj splicing) OR intein)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2007/05/11 17:01
L8	26	l7 and ((fluorescent adj protein) OR GFP)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2007/05/11 16:36
L9	27	watson and michnick.in.	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2007/05/11 16:26
L10	20	l9 and subcellular	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2007/05/11 16:58
L11	20	l10 and l1	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2007/05/11 16:27
L12	1	("6780599").PN.	USPAT	OR	OFF	2007/05/11 16:36
L13	12	l8 and (subcellular OR organelle OR targeting)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2007/05/11 16:58
S1	92	yoshio near2 umezawa.in.	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2007/05/09 13:03
S2	21	takeaki near2 ozawa.in.	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2007/05/11 11:44
S3	1	WO02/08766	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2007/05/11 11:44

FILE 'BIOSIS' ENTERED AT 17:14:14 ON 11 MAY 2007

=> s protein-protein interaction
L1 110247 PROTEIN-PROTEIN INTERACTION

=> s l1 (P) ((protein splicing) OR intein)
L2 62 L1 (P) ((PROTEIN SPLICING) OR INTEIN)

=> s l2 and ((fluorescent protein) OR gfp OR egfp)
L3 21 L2 AND ((FLUORESCENT PROTEIN) OR GFP OR EGFP)

=> s l3 and (subcellular OR organelle)
L4 1 L3 AND (SUBCELLULAR OR ORGANELLE)

AU Ozawa, Takeaki
SO Molecular Medicine (Tokyo, Japan) (2003), 40(11), 1384-1386
CODEN: MOLMEL; ISSN: 0918-6557
TI Mechanism of protein splicing and the role of intein
AB A review discussed the mechanism of protein splicing and the role of intein in the process. The mechanisms of the splicing off of the intein segment from the protein precursor and consequent functional restoration of the protein were described. The use of the fusion protein of N-terminal GFP (green fluorescent protein) plus intein plus C-terminal GFP as reporting system of subcellular events was described. The anal. of protein-protein interaction in subcellular compartments and screening of organelle-targeting proteins were described as application examples.

AU Ozawa T; Nogami S; Sato M; Ohya Y; Umezawa Y
SO Analytical chemistry, (2000 Nov 1) Vol. 72, No. 21, pp. 5151-7.
Journal code: 0370536. ISSN: 0003-2700.
TI A fluorescent indicator for detecting protein-protein interactions in vivo based on protein splicing
.
AB We describe a new method with general applicability for monitoring any protein-protein interaction in vivo. The principle is based on a protein splicing system, which involves a self-catalyzed excision of protein splicing elements, or inteins, from flanking polypeptide sequences, or exteins, leading to formation of a new protein in which the exteins are linked directly by a peptide bond. As the exteins, split N- and C-terminal halves of enhanced green fluorescent protein (EGFP) were used. When a single peptide consisting of an intein derived from Saccharomyces cerevisiae intervening the split EGFP was expressed in Escherichia coli, the two external regions of EGFP were ligated, thereby forming the EGFP corresponding fluorophore. Genetic alteration of the intein, which involved large deletion of the central region encoding 104 amino acids, was performed. In the expression of the residual N- and C-terminal intein fragments each fused to the split EGFP exteins, the splicing in trans did not proceed. However, upon coexpression of calmodulin and its target peptide M13, each connected to the N- and C-terminal inteins, fluorescence of EGFP was observed. These results demonstrate that interaction of calmodulin and M13 triggers the refolding of intein, which induces the protein splicing, thereby folding the ligated extein correctly for yielding the EGFP fluorophore. This method opens a new way not only to screen protein-protein interactions

but also to visualize the interaction in vivo in transgenic animals.

AU Ozawa T; Umezawa Y
SO Current opinion in chemical biology, (2001 Oct) Vol. 5, No. 5, pp. 578-83.
Ref: 36
Journal code: 9811312. ISSN: 1367-5931.
TI Detection of protein-protein interactions in vivo based on protein splicing.
AB In mammalian cells, protein-protein interactions constitute essential regulatory steps that modulate the activity of signaling pathways. In recent years, several approaches towards understanding the interactions have been developed. We describe herein a new method for detecting protein-protein interactions in vivo based on protein splicing and highlight some potential applications of this technique.

AU Ozawa T; Takeuchi T M; Kaihara A; Sato M; Umezawa Y
SO Analytical chemistry, (2001 Dec 15) Vol. 73, No. 24, pp. 5866-74.
Journal code: 0370536. ISSN: 0003-2700.
TI Protein splicing-based reconstitution of split green fluorescent protein for monitoring protein-protein interactions in bacteria: improved sensitivity and reduced screening time.
AB In this research, an improved detection system is described that allows an easy in vivo screening and selection of functional interactions between two interacting proteins in bacteria. We earlier reported a new concept for detecting protein-protein interactions based on reconstitution of split-enhanced green fluorescent protein (EGFP) by protein splicing (Ozawa, T.; et al. Anal. Chem. 2000, 72, 5151-5157.): Two putative interacting proteins are genetically fused to the split VDE inteins, which are linked directly to the N- and C-terminal halves of the split EGFP. Association of the interacting proteins results in functional complementation of VDE and protein-splicing reaction that leads to formation of an EGFP fluorophore. This technique simplified detection of protein interactions, but because of the low splicing efficiency of VDE intein, its sensitivity and screening time were not enough for detecting the protein interactions directly in living cells. In this paper, we have explored the use of the DnaE split intein from Synechocystis sp. PCC6803 for intracellular reconstitution of the split EGFP. We examined efficiency of the fluorophore formation by preparing four different split-EGFP types, among which EGFP dissected at the position between 157 and 158 was found to show the strongest fluorescence intensity upon protein interactions. A time required for the formation of EGFP after protein interactions was only 4 h, as compared to 3 days with the VDE intein. The protein interactions were thereby detected by an in vivo selection and screening assay in Escherichia coli on Luria broth agar plates. This improvement permits versatile designs of screening procedures either for ligands that bind to particular proteins or for molecules or mutations that block particular interactions between two proteins of interest.

AU Kanno, Akira; Ozawa, Takeaki; Umezawa, Yoshio
SO Analytical Chemistry (2006), 78(2), 556-560
CODEN: ANCHAM; ISSN: 0003-2700
TI Intein-mediated reporter gene assay for detecting protein-protein interactions in living mammalian cells
AB For nondestructive anal. of chem. processes in living mammalian cells, here we developed a new reporter gene assay for detecting cytosolic protein-protein interactions based on protein splicing of transcription factors with DnaE

inteins. The protein splicing induces connection of a DNA-binding protein (modified LexA; mLexA) with a transcription activation domain of a herpes simplex virus protein (VP16AD). We thereby circumvented the limitation of earlier methods for monitoring protein-protein interactions, including the two-hybrid systems, protein complementation systems (PCS), and protein reconstitution systems, and rather combined their advantages. To test the applicability of this method, we monitored epidermal growth factor (EGF)-induced interactions on cell membranes of a known partner, an oncogenic product Ras and its target Raf-1. Ras was connected with N-terminal DnaE and mLexA, while Raf-1 was connected with C-terminal DnaE and VP16AD. Upon stimulation with EGF, the interaction between Ras and Raf-1 triggered folding of the DnaEs, thereby inducing protein splicing to form mLexA-VP16AD fusion protein, and transcription of a reporter gene, firefly luciferase. The extent of Ras-Raf-1 interaction was quantified by measuring the luciferase activity. The interaction was not able to be monitored by two-hybrid systems nor by PCS of split firefly luciferases; however, by using the protein splicing elements and the reporter gene, we obtained the bioluminescence signals sufficient for evaluation of the interactions close to cell membranes.